

Is brain estradiol a hormone or a neurotransmitter?

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Mounting evidence indicates that, besides their well-known hormonal mode of action at the genetic level, estrogens such as 17 β -estradiol also influence brain function by direct effects on neuronal membranes. Experimentally induced rapid changes in estradiol bioavailability in the brain have been shown to alter the expression of male sexual behavior significantly within minutes – probably too quickly to be accounted for by conventional genetic mechanisms. In parallel, recent studies indicate that aromatase, the enzyme that converts testosterone to estradiol in the brain, is expressed in presynaptic terminals and modulated within minutes by Ca²⁺-dependent phosphorylation. In this article, we develop the hypothesis that brain estrogens display many, if not all, functional characteristics of neuromodulators or even neurotransmitters.

Introduction: neurotransmitters versus hormones

Chemical communication between cells is fundamental to neural function. Categorizing chemical messengers present in the brain is necessary when trying to organize the seemingly endless complexity of the CNS. One categorical distinction established nearly 100 years ago is that between hormones and neurotransmitters [1], but the validity of this distinction continues to be challenged. Our focus here is on the steroid hormone 17 β -estradiol (E₂) and its identity as a chemical messenger. Hormones are defined traditionally as compounds that are released into the blood from endocrine glands and act at distant target sites. They are thought to induce biological effects via a relatively slow mode of action after binding to membrane or intracellular receptors. Neurotransmitters, by contrast, are chemical messengers that are synthesized by neurons, are stored in presynaptic vesicles in boutons and act relatively rapidly via selective postsynaptic receptor proteins. Specific enzymes or reuptake proteins rapidly terminate their action. The discovery of neurohypophyseal hormones in the 1950s challenged the distinction between these categories. Peptides such as oxytocin and vasopressin are synthesized by neurons in the brain but then released either into the brain or directly into the blood, enabling them to act at distant targets [1]. Novel messengers such as the free radical gases nitric oxide and carbon monoxide also raise questions about the classification of chemical messengers. These are not stored in

vesicles and diffuse to target cells where they act on ‘enzyme receptors’ such as guanylyl cyclase. Thus they possess some traits in common with neurotransmitters but lack others, leading Snyder and Ferris [2] to propose that the concept of neurotransmission be liberalized, such that ‘A transmitter is a molecule, released by neurons or glia that physiologically influences the electrochemical state of adjacent cells’. Recent work on brain steroids, in particular estrogens synthesized by local aromatization of androgenic steroids, reveals that E₂ in the brain displays many features traditionally associated with neurotransmitters. We present here a selective review of the recent literature on rapid effects of estrogens on behavior and physiology, in addition to data demonstrating rapid changes in the production of estrogens in the brain that support this contention.

Fast actions of estrogens on cell function

It has been known for many years that estrogens such as E₂ mediate some of their effects via nuclear receptors that act as transcription factors to modulate gene transcription, and that they also mediate other, more rapid effects in the brain that do not involve genomic mechanisms (or do so only indirectly) [3–5]. For example, estrogens can change the electrophysiological activity of various populations of neurons with latencies of a few seconds [6], or reduce within minutes Ca²⁺ currents in brain areas that do not seem to express nuclear estrogen receptors [7]. This leads to activation of specific protein kinase pathways that can affect gene expression indirectly, including the mitogen-activated protein kinase (MAPK) pathway and those involving cAMP, such as the protein kinase A (PKA) and the cAMP-response-element-binding protein (CREB) pathways [5,8,9]. Although it is clear that the brain has estrogen-sensitive transducers that can support the action of estrogens within minutes or even seconds and milliseconds, some of these effects cannot be implemented in a temporally relevant fashion unless there are mechanisms for rapidly modifying (increasing and decreasing) the local bioavailability of estrogens [10]. The traditional notion that estradiol synthesis in the ovary, and thus the circulating estradiol concentrations, change over only relatively long time periods (hours to days, from one steady-state situation to another) has discouraged the investigation of more rapid regulatory mechanisms. It is unlikely that changes in the synthesis of estrogens as implemented in the periphery (ovaries or

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adipose tissue) would be dynamic enough to explain rapid physiological responses in the brain.

Fast versus slow regulation of brain estrogen synthase *Control of aromatase transcription in the brain by steroids*

It was discovered in the early 1970s that estrogens are synthesized in the brain by aromatization of androgens such as testosterone [11]. Estrogens locally produced in the preoptic area have a key role in the activation of sexual behavior in various vertebrate species [12]. Preoptic aromatase activity is, in contrast to aromatase expressed in ovarian or adipose tissues, regulated by genomic actions of sex steroids [13,14]. Testosterone treatment of castrated birds [15,16] and mammals [17] markedly increases aromatase activity in the preoptic area within a few days.

Testosterone similarly increases the concentration of aromatase protein and corresponding mRNA, suggesting that the testosterone-induced changes in preoptic aromatase activity are largely the result of an increase in gene transcription [18,19]. These effects of testosterone on aromatase transcription are mediated (and can be mimicked) by a synergistic action of the androgenic (e.g. 5α -dihydrotestosterone) and estrogenic (e.g. E_2) metabolites of this steroid [19]. However, such changes in aromatase activity that are induced by genomic effects of steroid hormones are much too slow (hours to days in duration) to produce rapid changes in E_2 bioavailability that could trigger the membrane effects of the steroid (which are detectable in minutes). Is aromatase regulated in other ways that might be consistent with the time course of the membrane effects of steroid hormones?

Fast changes in aromatase activity following phosphorylation of the enzyme

Recent research indicates that, as observed for many other enzymes [20], aromatase activity is markedly affected by Ca^{2+} -dependent phosphorylation. Profound inhibition of enzymatic activity is observed in homogenates of the quail (*Coturnix japonica*) preoptic area that have been preincubated with increased but physiological concentrations of ATP, Mg^{2+} and Ca^{2+} [21]. This inhibition is blocked by agents that chelate divalent ions such as EGTA or EDTA (Figure 1a) or by kinases inhibitors (Figures 1b,d), in particular inhibitors of PKA or protein kinase C (PKC) but not those of protein kinase G or calmodulin kinase II, indicating that the inhibition is indeed caused by phosphorylation processes [21,22].

The predicted quail aromatase sequence contains multiple (15) phosphorylation consensus sites, including two (Thr455 and Thr486) corresponding to the specificity of PKC and/or PKA [22]. Western blotting on quail brain aromatase partially purified by immunoprecipitation further demonstrates that phosphorylation sites on the aromatase protein are affected by phosphorylating conditions (i.e. presence of EGTA or of ATP, Mg^{2+} and Ca^{2+} ; Figure 1c) that influence the enzymatic activity [22,23]. From these findings we can conclude that, in brain homogenates, aromatase activity can be regulated by Ca^{2+} -dependent phosphorylation much more rapidly than by changes in concentration of the enzyme, which was

previously the only mechanism known to affect brain aromatase activity. However, do these regulatory processes occur in intact neural systems? Subsequent experiments therefore investigated whether this mechanism identified *in vitro* has physiological relevance.

Fast regulation of aromatase activity by afferent inputs
Changes in intracellular Ca^{2+} concentrations also drastically modify aromatase activity in preoptic-hypothalamic explants that are maintained *in vitro* in a medium containing [$1\beta^3$ -H]-androstenedione, which releases tritiated water during aromatization, so that aromatase activity can be measured every 5 min [21]. K^+ -induced depolarization (which enables Ca^{2+} to enter the cells freely) or exposure to thapsigargin (a lactone that mobilizes intracellular pools of Ca^{2+}) rapidly and reversibly inhibits the enzymatic activity [21] (Figure 1e). It is highly probable that this regulation is even faster than already reported, but that this cannot be detected owing to experimental limitations (see [21,24] for discussion).

Similar inhibitions of aromatase activity are observed in these explants following stimulation by the glutamatergic agonists kainate (Figure 1f) or AMPA, but not NMDA. These effects are reversible and are blocked by preincubation with antagonists specific to AMPA and kainate receptors [24] (Figure 1g). Electrophysiological studies indicate that aromatase-immunoreactive cells are sensitive to dopamine, AMPA, kainate and NMDA, making it probable that these compounds are acting directly on aromatase-expressing cells [25,26]. These electrophysiological studies also demonstrate the existence of a tonic electrical activity in the preoptic area of quail [25,26] and rat [27] that can be blocked by antagonists of glutamate or GABA receptors. Because (i) aromatase activity is rapidly inhibited by glutamate and (ii) tonic glutamatergic inputs seem to be present in the preoptic area and are modulated (in rats) by copulatory activity [28], it is likely that aromatase activity is chronically maintained at sub-maximal levels *in vivo* by these inputs, and can thus both increase and decrease as a function of changes in glutamate activity. The increase in glutamate activity during copulation, if it occurs in quail as it does in rats, would also explain why preoptic aromatase activity decreases in male quail within 1–5 min after copulation [29]. Irrespective of the specific cellular mechanism or mechanisms that are involved, these data clearly show that production of estrogens by the brain can vary with latencies of minutes or possibly seconds in response to changes in neurotransmitter (especially glutamate) activity.

Cellular localization of aromatase and its physiological significance

Aromatase-expressing cells are distributed in a relatively small number of discrete brain regions primarily in the diencephalon. However, fibers originating from these cells are much more widespread and can be detected in a relatively large number of brain areas [30,31]. Within cells, a significant fraction of aromatase activity appears to be located in presynaptic boutons. This enzymatic activity is enriched in synaptosomes prepared from zebra

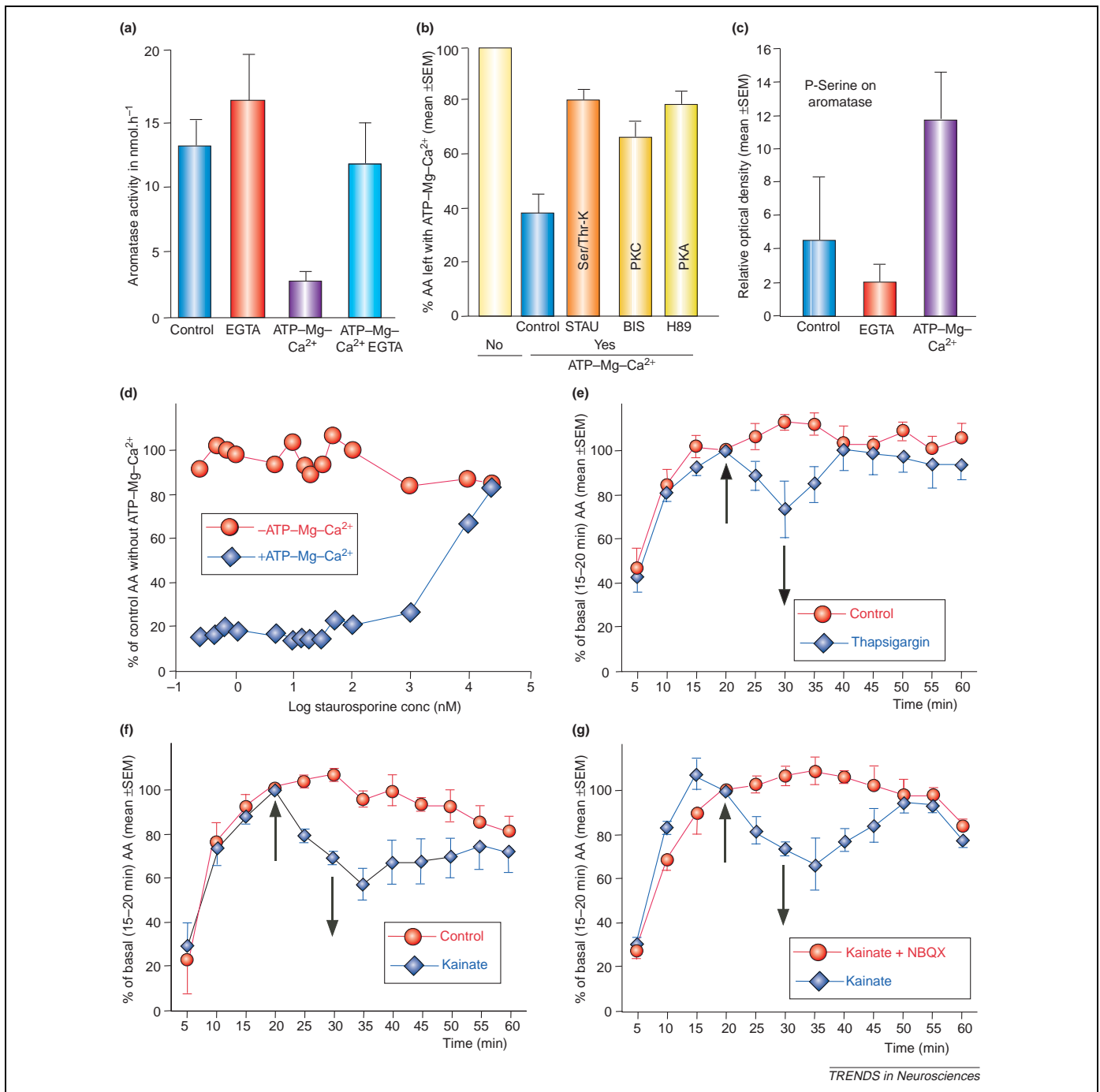


Figure 1. Summary of experimental evidence demonstrating that aromatase activity can be rapidly modulated by Ca²⁺-dependent phosphorylation in quail brain homogenate (a-d) and by afferent inputs presumably modulating intracellular Ca²⁺ levels in preoptic-hypothalamic explants (e-g). (a) Aromatase activity is drastically decreased by a 15-min pre-incubation of hypothalamic homogenates in the presence of ATP, Mg²⁺ and Ca²⁺, and this inhibition is completely blocked by a Ca²⁺-chelating agent such as EGTA. (b) The decrease in aromatase activity (AA) induced by pre-incubation with ATP, Mg²⁺ and Ca²⁺ is largely blocked in presence of the serine/threonine kinase inhibitor staurosporine (STAU), the protein kinase C (PKC) inhibitor bisindolylmaleimide (BIS), or the protein kinase A (PKA) inhibitor H89. (c) Quantification by western blot analysis of serine phosphate on aromatase purified by immunoprecipitation. Preincubation of hypothalamic homogenates with ATP, Mg²⁺ and Ca²⁺ (i.e. stimulation of phosphorylation) increases the amount of serine phosphate on the aromatase band as compared with homogenates incubated with 1 mM EGTA (i.e. inhibition of phosphorylation). Serine phosphorylation in control conditions is intermediate and not significantly different from the EGTA condition. (d) Effect of increasing doses of the protein kinase inhibitor staurosporine on aromatase activity measured in hypothalamic homogenates in the presence or absence of ATP, Mg²⁺ and Ca²⁺. Data are expressed as percentages of the enzymatic activity observed in the absence of inhibitor and of ATP-Mg²⁺-Ca²⁺. (e) Aromatase activity in paired hypothalamic explants incubated *in vitro* in which one explant was exposed for 10 min [between 20 min (upward arrow) and 30 min (downward arrow)] to thapsigargin, a lactone that mobilizes intracellular Ca²⁺ stores. All data are expressed as percentages of basal release, defined as the activity during the period preceding the experimental manipulation (15–20 min). (f) Aromatase activity in paired hypothalamic explants incubated *in vitro*; one explant was exposed for 10 min [between 20 min (upward arrow) and 30 min (downward arrow)] to the glutamate agonist kainate (100 μM). All data are expressed in percentage of basal release, defined as the activity during the period preceding the experimental manipulation (15–20 min). (g) Aromatase activity in paired hypothalamic explants incubated *in vitro* in which both explants were exposed for 10 min [between 20 min (upward arrow) and 30 min (downward arrow)] to the glutamate agonist kainate (100 μM); one explant was first pre-incubated with the non-NMDA glutamate antagonist NBQX. All data are means ± SEM. Redrawn from data in [19,22,24].

finch or rat brain [32,33], and dense aromatase immunoreactivity is observed in presynaptic boutons at the surface of synaptic vesicles [34–36]. Recent western blot studies also indicate that aromatase expression is differentially controlled in the microsomal and synaptosomal compartments [37]. This anatomical organization, in conjunction with the rapid phosphorylation-mediated changes in aromatase activity, strongly indicates that rapid changes in estradiol bioavailability occur in synapses. Aromatizable androgens derived from the peripheral circulation or possibly from local *de novo* synthesis [38] are freely available in the brain; this local aromatization should result in the presence of particularly high concentrations of estrogens presynaptically, which could potentially activate membrane responses that are insensitive to the lower concentrations of estrogens derived from the peripheral circulation. This situation is similar to that described for the activation of GABA_A receptors by progestins [39].

Furthermore, there is now evidence that some estrogen-dependent aspects of hippocampal plasticity cannot be stimulated in rats by peripheral concentrations of estrogens. For example, the addition of the aromatase inhibitor letrozole markedly reduces expression of the synaptic protein synaptophysin in hippocampal cell cultures derived from male or female rats. This inhibition, caused by the loss of local estrogen production, cannot be rescued by treating cultures with estradiol concentrations that mimic the peripheral concentrations present in males (10^{-12} M) or in females (10^{-10} M). Much higher concentrations (10^{-7} M) are required to reverse this effect of aromatase inhibition [40], supporting the notion that production of estrogens in the brain via local aromatization of testosterone is necessary to implement certain estrogen-dependent responses. This scenario is probably true for both males and females given that, in many species, females display circulating concentrations of testosterone (the substrate of aromatase) that are clearly higher than those of estradiol (e.g. in quail plasma, testosterone concentrations are ~ 1500 pg ml⁻¹ and ~ 500 pg ml⁻¹ in males and females, respectively, whereas concentrations of estradiol do not exceed 100 pg ml⁻¹ in males and 300 pg ml⁻¹ in females [41]). Evidence that such high concentrations of estradiol are localized in the synapses and are functionally relevant is important, but alone this does not support a transmitter-like action for estrogens.

Fast effects of E₂ on behavior and physiology

Effects on appetitive and consummatory aspects of male sexual behavior

Although rapid effects of estrogens on cell function have been widely identified, few studies have investigated rapid E₂ effects on whole-organism responses in behavior and physiology. One study on rats identified effects of estradiol injections on male sexual behavior after latencies of 20–30 min [42]. Rapid membrane actions of E₂ were also shown to enhance the genomic effects of this steroid on lordosis behavior (i.e. taking up a sexually receptive position that allows mounting and intromission by the male) in female rats [43], in addition to

the aforementioned multitude of rapid effects of E₂ that have been described at a cellular level. We thus hypothesized that the rapid changes in brain aromatase activity identified in the quail experiments summarized in the preceding section would have a significant impact on the expression of sexual behavior in male quail.

Castrated quail treated with a small behaviorally ineffective dose of testosterone (a 2-mm-long Silastic™ implant filled with crystalline testosterone) were injected intraperitoneally with a bolus of E₂ to mimic a rapid increase of estrogens in the brain (Figure 2a). When tested 15–60 min later, these birds displayed, in comparison with vehicle-injected control birds, significant increases in the occurrence and frequency of copulatory behavior 15 min after injection. However, there was no longer a statistically significant effect of E₂ when birds were tested 30 or 45 min after the E₂ injection [44].

Conversely, we mimicked the effects of a decrease in estrogens, as would be observed following the rapid inhibition of aromatase activity by Ca²⁺-dependent phosphorylation, by systemically injecting sexually active males (gonad-intact birds, or castrated animals treated using 40-mm-long testosterone implants) with a large dose of an aromatase inhibitor (Figure 2b). A single injection of the aromatase inhibitor Vorozole™ significantly reduced most aspects of male copulatory behavior. Maximal effects were observed 30–45 min after injection [45]. This behavioral result is fully consistent with the observation that a similar injection completely suppresses brain aromatase activity measured 30 min post injection [45]. We also found, using the preoptic explant preparation previously described, that the addition of Vorozole™ completely blocks aromatase activity within < 15 min [45].

Acute inhibition of aromatase activity following Vorozole™ injection also decreased male appetitive sexual behavior after 30 min [45], as assessed by the frequency of rhythmic cloacal sphincter movements (RCSM) [46,47] (Figure 2c) or by the expression of the learned social proximity response [48,49] – two sexually motivated responses that had been shown previously to be activated by estrogens derived from aromatization of testosterone in the brain [12,47]. Inhibition of RCSM was still significant after 45 min but it was no longer statistically significant after 60 min, and behavior returned to control levels on the next day [45]. Injection of another aromatase inhibitor, androstatrienedione, induced a similar rapid inhibition of sexually-motivated cloacal sphincter movements [45].

Pain thresholds

Estrogens are well known to regulate pain thresholds over long time periods (e.g. [50–52]). However, recently it has been established in quail that there are also rapid effects of estrogen on responses to painful stimuli. Aromatase is expressed and is enzymatically active in pain-sensitive neurons in the dorsal horn of the quail spinal cord [53]. Locally produced estrogens appear to modulate pain responses to noxious stimuli rapidly. Castration markedly increases the latency of foot withdrawal from a 54°C hot water bath, whereas chronic testosterone treatment decreases this latency, by the action of its estrogenic

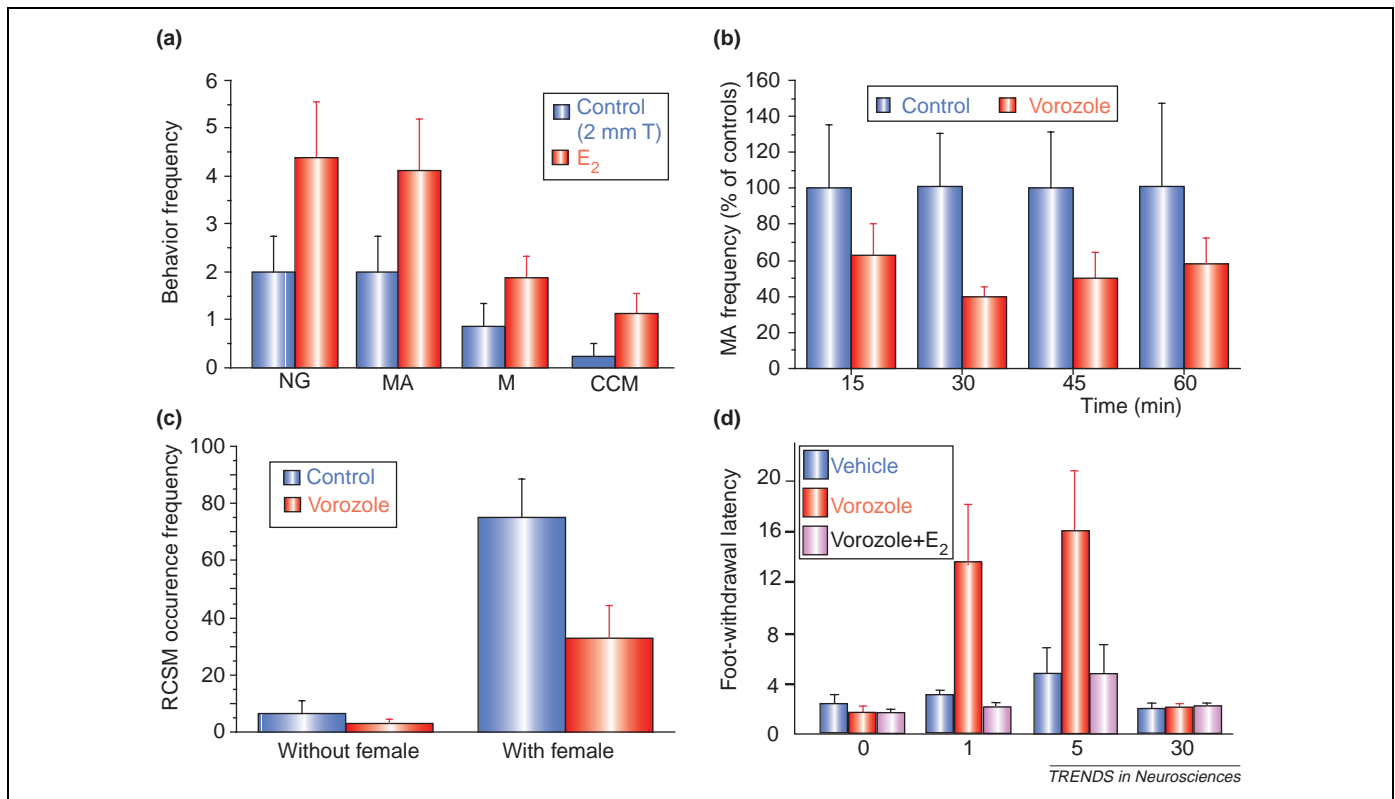


Figure 2. Examples illustrating the effects of rapid changes in the availability of estrogens within the CNS on sex behavior and on responses to noxious stimuli in male quail. **(a)** In castrated males treated with a very small dose of testosterone (2 mm Silastic™ implants) that does not activate an intense copulatory behavior by itself, the injection of E₂ (500 μg kg⁻¹) 15 min before testing significantly increases all aspects of copulatory behavior compared with controls. Abbreviations: CCM, cloacal contact movements; M, mounts; MA, mount attempts; NG, neck grabs. **(b)** Injection of Vorozole™ to gonad-intact sexually active males decreases copulatory behavior (mount attempts). Maximal effects are observed 30–45 min after injection. All data are normalized as percentages of the behavioral frequencies observed in control subjects at each latency after injection. **(c)** A view of a female drastically increases the frequency of rhythmic cloacal sphincter movements (RCSM) in gonad-intact sexually active males, and this effect is significantly inhibited by Vorozole™ injected 30 min before testing. **(d)** Rapid, presumably non-genomic, effects of changes in the bioavailability of estrogens in the spinal cord on the foot-withdrawal latency in the hot-water test (54°C) in sexually mature gonad-intact male quail. Mimicking a decrease in E₂ production, as might occur following aromatase phosphorylation, by an intrathecal injection of the aromatase inhibitor Vorozole™ increases the foot-withdrawal latency within 1 min and latency is back to normal after 30 min. The effect is blocked by the co-injection of a bolus of E₂. Redrawn from data in [44,45,55].

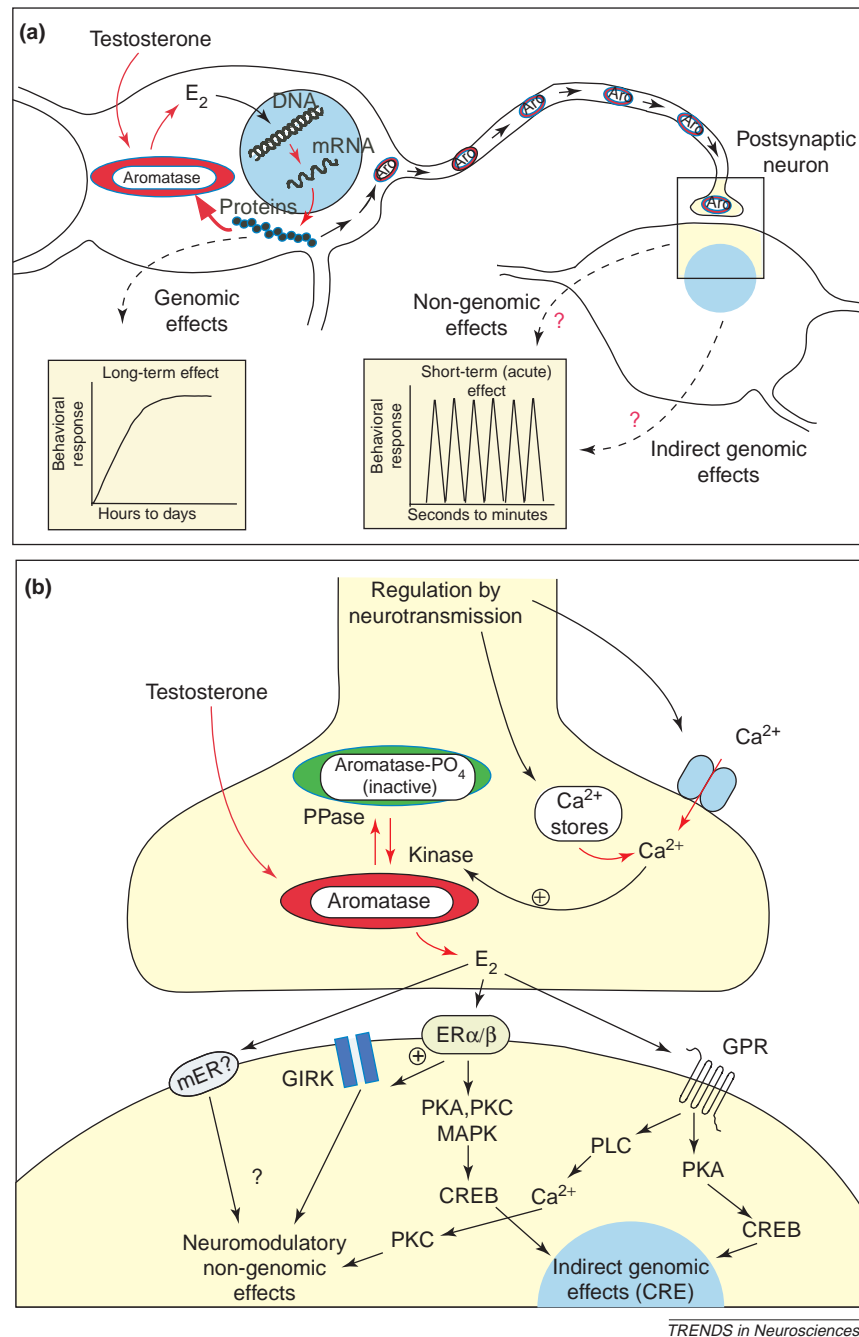
metabolites [54]. Highly significant increases of foot-withdrawal latency in the hot-water test are also observed within 1–5 min after acute intrathecal injections of aromatase inhibitors at the lumbar level [55]. The effect of acutely inhibiting estrogen production in the quail spinal cord (by systemic injection of Vorozole™ or androstatrienedione) on the behavioral responsiveness to painful thermal stimuli is completely blocked by concurrent injection of E₂ (Figure 2d). Similar effects were recently reported in rats [56]. Rapid effects of locally produced estrogens on neuronal physiology and behavior are thus apparent in the spinal cord in addition to the brain. Overall, these data support the notion that rapid changes in E₂ production in the brain and spinal cord have functional consequences for whole-organism responses.

What is the cellular basis for these rapid effects on behavior?

During the past decade, E₂ has been shown to have short-latency effects on many cellular and biochemical events associated with various neural messenger systems. This topic has been reviewed extensively (e.g. [5,6,57,58]) and is beyond the scope of this paper. The mechanisms mediating these cellular actions of estrogens are good candidates to explain the behavioral effects already described. For example, rapid changes in E₂ availability

in neuronal perikarya could modulate the activity of various kinases (e.g. PKA, PKC, MAPK or phosphatidylinositol-3 kinase) [59,60], thus affecting phosphorylation of various molecules implicated in second messenger signaling cascades (e.g. CREB [8,61]) or in neurotransmission (e.g. tyrosine hydroxylase phosphorylation [62]).

Given that aromatase is present in presynaptic boutons, changes in E₂ release in synapses might also act on the postsynaptic membrane, through an interaction with GABA or glutamate receptors, and/or with one or several of the putative membrane estrogen receptors (or estrogen-sensitive systems) that have been tentatively identified. These include (i) intracellular nuclear estrogen receptors (i.e. α and β subtypes) that can become associated with the cell membrane, or receptors of a different nature that at least show specific immune cross-reactivity with the nuclear ones, (ii) forms of receptors associated with membrane caveolae, (iii) the newly identified ER-X receptor, and (iv) other novel membrane estrogen receptors, including G-protein-coupled seven-transmembrane-domain receptors such as GPR30 [57,58] (Figure 3). There are therefore multiple transduction systems for fast signaling by estrogens in the neuronal perikarya or at the membrane level, and future research will need to evaluate the specific role of these different proteins.



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Figure 3. Mechanisms through which rapid changes in estrogen production presynaptically, caused by phosphorylation and dephosphorylation of the aromatase enzyme, might modulate neuronal activity in a manner similar to the action of a neurotransmitter or neuromodulator. **(a)** In the aromatase-expressing neuron, aromatase transcription is enhanced by the action of testosterone or its aromatized metabolite, estradiol (E₂). Steroid action also results in the transcription of various other proteins that modify neural activity in the long term and have consequently long-lasting (e.g. seasonal) effects on reproductive behaviors. Aromatase is then transported to presynaptic boutons, as demonstrated by immunocytochemical studies at the electron-microscopic level, where the enzyme catalyzes the transformation of testosterone into E₂. Red arrows represent metabolic pathways or actual movements of compounds, and black arrows represent control mechanisms. **(b)** Changes in intracellular Ca²⁺ levels, as triggered by afferent glutamatergic inputs or other aspects of neurotransmission, regulate aromatase activity by controlling its phosphorylation [i.e. modulation of kinase and phosphatase (PPase) activities]. Panel (b) represents a higher magnification of the boxed area in (a). E₂ released at the presynaptic level then affects the activity of the postsynaptic membrane by interacting with various estrogen-sensitive 'receptor' proteins such as the nuclear estrogen receptors α or β (ER α/β) associated with the membrane, or G-protein-coupled receptors (GPR), or other forms of membrane estrogen receptor still to be identified (mER?). Activation of these proteins affects intracellular signaling by modifying activity of inwardly rectifying K⁺ channels (GIRK), modulating Ca²⁺ levels [e.g. through activation of phospholipase C (PLC)] or by changing the activity of various kinases [protein kinase A (PKA), protein kinase C (PKC), mitogen-activated protein kinase (MAPK)], resulting in the phosphorylation of various proteins [e.g. cAMP-responsive element-binding protein (CREB)]. Phosphorylated proteins then bind to specific response elements to induce indirect genomic effects (e.g. pCREB binding to the CREB-response element CRE), or alternatively neuronal activity is rapidly affected by non-genomic effects. All responses to the rapid changes in E₂ concentrations have been shown at the postsynaptic level in a neuron that does not express aromatase by itself; however, most of these effects might also take place in the perikaryon of the aromatase-expressing neuron (not shown here, for simplicity). Red arrows represent metabolic pathways or actual movements of compounds, and black arrows represent control mechanisms.

Does E₂ meet the criteria for a neurotransmitter, and why is that important?

The concept of neurosecretion and associated ideas about the unity of action of the nervous and endocrine systems have been articulated in some form for decades, but assumptions that endocrine and neural signaling are respectively mediated by hormones and neurotransmitters can hinder scientific thinking. Subsequent to the discovery of neurosecretion, it was established that some hormones, in particular testosterone, are actually pro-hormones that must be transformed at target sites into more effective metabolites such as E₂ [11,63]. The identification of neurosteroids (steroids synthesized *de novo* by the brain, potentially including E₂) led to the notion that the brain can itself synthesize steroids for use as messengers [39,64].

Based on experimental evidence summarized here, we argue that estrogens produced locally in the brain through aromatization of testosterone, or of other androgenic substrates eventually produced in the brain from cholesterol, fulfill most if not all criteria for a neurotransmitter, or at least a neuromodulator (i.e. a substance that influences neurotransmission without acting as a transmitter itself, usually on a timescale of minutes to hours [65,66]). It is indeed established that:

- (i) E₂ is produced in neurons by enzymatic transformations that are rapidly (within minutes, or possibly seconds) modulated by Ca²⁺-dependent phosphorylation under the influence of glutamatergic inputs [21,22,24]. Importantly, E₂ synthesis occurs, at least in part, in presynaptic boutons [32–36]. Rapidly changing high concentrations of E₂ are thus likely to be produced in neurons, and might control both intracellular signaling cascades and activity of the postsynaptic membrane.
- (ii) Besides their rapid actions at the cellular level (e.g. [3,4,6,9,60]), which are beyond the scope of this article, rapid changes in local E₂ bioavailability have a significant impact on various behavioral and physiological responses, including activation of male [42,44,45] and possibly female [43] behavior and latency of reactions to noxious stimuli [55]. We have focused here on the behavioral effects of estrogens that can be observed with latencies of a few minutes but many studies have also reported cellular effects of estrogens in the brain and other target structures that can be observed with even shorter latencies. As already mentioned, multiple cellular and biochemical mechanisms have been identified that could mediate these behavioral effects, and an obvious goal of future research is to identify the specific cellular mechanisms underlying each effect.
- (iii) Inactivation mechanisms are present in the brain to terminate estrogen signaling when production of the steroid is turned off by inhibition of aromatase (via phosphorylation). The observation that a single injection of an aromatase inhibitor rapidly affects male quail sexual behavior and responses to noxious stimuli [45,55] indeed implies that locally

produced estrogens should rapidly be cleared from the tissue as soon as aromatase activity is interrupted. In the periphery, estrogens are catabolized to hormonally inactive (or less active) water-soluble metabolites by oxidative metabolism (largely through 2- and 4-hydroxylation by cytochrome P450 enzymes, and through glucuronidation, sulfonation and/or O-methylation; see [45] for references). Metabolism of estrogens in the brain has received less attention but detectable levels of the relevant catabolic enzymes have been identified in the brains of various species. These include: 2- and 4-hydroxylases [67–69]; catecholamine-O-methyltransferases (COMTs), which transform catecholestrogens into methoxyestrogens that have a weaker hormonal activity [70]; and glucuronidase and sulfotransferase [71–73]. Interestingly, aromatase seems to catalyze both the aromatization of testosterone into E₂ and the 2-hydroxylation of estrogens into catecholestrogens, and it seems to act as an estrogen synthase or as catecholestrogen-forming enzyme as a function of the available substrate and local pH [74]; this should provide an effective method for rapidly controlling local concentrations of bioactive estrogens. Although more work is needed on this topic, these data strongly suggest that the synthesis of estrogens and catabolism by hydroxylation might proceed at similar rates, thus producing symmetrical upregulation and downregulation of the local concentration of this steroid. Alternatively, diffusion might rapidly equilibrate the potentially high brain local concentration of E₂ produced by local aromatization with peripheral concentrations; such levels were shown in at least one model system to be too low to activate some brain responses triggered by locally produced estrogens [40]. This represents an alternative mechanism for rapid termination of the action of locally produced estrogens.

Concluding remarks

The recently identified rapid changes in aromatase activity address in part the conceptual gap between the well-documented rapid non-genomic effects of estrogens in the brain and the apparent lack of mechanisms to modify estrogen availability rapidly in specific brain areas [10]. Experiments mimicking the activation or inactivation of aromatase, by acute injection of E₂ or an aromatase inhibitor respectively, rapidly affect the expression of sexual behavior or the reaction to noxious stimuli, thereby indicating that rapid changes in the bioavailability of estrogens do have functional significance. Locally produced brain estrogens should therefore not only be considered as neuroactive steroids; they also display many (if not all) of the functional characteristics of neuromodulators and possibly neurotransmitters. In all probability, such actions would be more akin to the action of neuromodulators such as neuropeptides and catecholamine transmitters, which act relatively slowly via metabotropic receptors, than to amino acids, which act quickly and directly on ion channel receptors.

This distinction is more than just of semantic interest. Aspects of steroid hormone action in the brain (including actions of locally produced estrogens) should be viewed as another neural signaling system that acts rapidly, is fine-tuned in response to environmental and experiential changes (namely via afferent glutamatergic inputs to aromatase-expressing neurons), and is essential for the regulation of specific behavioral systems. The discovery that gases such as nitric oxide can act as chemical messengers in the brain has challenged traditional notions about what constitutes a neurotransmitter [2,75]. Recent findings on the actions of estrogens in the brain reviewed here are adding to our understanding of the diversity of substances that act as neurochemical messengers and can modify the functioning of adjacent cells in significant ways. These concepts will have a major impact on planning future research programs and designing clinical interventions that involve actions of estrogen in the brain.

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